

Application No. 10/658,609  
Second Suppl. Preliminary Amend. dated September 8, 2005

**Amendments to the Claims:**

- 1-68. (Canceled)
69. (Previously Presented) A method of depositing elemental metal in the vicinity of an enzyme, comprising:  
combining an enzyme with metal ions, an oxidizing agent and a reducing agent;  
incubating the enzyme with the metal ions in the presence of the oxidizing agent and the reducing agent, whereby the metal ions are reduced to elemental metal; and  
depositing the elemental metal in the vicinity of the enzyme, wherein the metal ions are selected from the group consisting of silver, gold, iron, mercury, nickel, copper, platinum, palladium, cobalt, iridium ions and a mixture thereof.
70. (Previously Presented) The method of claim 69, wherein the metal ions are silver ions.
71. (Previously Presented) The method of claim 69, wherein the metal ions are silver ions in a solution of silver acetate.
72. (Previously Presented) The method of claim 69, wherein the enzyme is an oxido-reductase.
73. (Previously Presented) The method of claim 69, wherein the enzyme is peroxidase.
74. (Previously Presented) The method of claim 69, wherein the enzyme is horseradish peroxidase.
75. (Previously Presented) The method of claim 69, wherein the enzyme is conjugated to streptavidin.
76. (Previously Presented) The method of claim 69, wherein the enzyme is conjugated to an antibody.

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77. (Previously Presented) The method of claim 69, wherein the oxidizing agent is an oxygen-containing oxidizing agent.

78. (Previously Presented) The method of claim 69, wherein the reducing agent is selected from the group consisting of hydroquinone, a hydroquinone derivative, and n-propyl gallate.

79. (Previously Presented) The method of claim 69, wherein the metal ions act as a substrate for the enzyme.

80. (Previously Presented) The method of claim 79, wherein the enzyme is peroxidase and the metal ions are incubated with the peroxidase in the absence of an organic substrate of the peroxidase.

81. (Previously Presented) The method of claim 80, wherein the organic substrate is a colorless organic substrate capable of being converted by the peroxidase to a colored substrate.

82. (Previously Presented) The method of claim 81, wherein the colorless organic substrate is 3,3'-diaminobenzidine or 5-bromo-4-chloro-3-indolyl phosphate.

83. (Previously Presented) The method of claim 69, wherein the step of incubating includes incubating the enzyme with the metal ions; adding the oxidizing agent and reducing agent to the mixture of the enzyme and the metal ions; and incubating the enzyme with the metal ions in the presence of the oxidizing agent and the reducing agent.

84. (Previously Presented) The method of claim 69, wherein the step of incubating includes incubating the enzyme with the metal ions; adding the reducing agent to the mixture of the enzyme and the metal ions;

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adding the oxidizing agent to the mixture of the enzyme, the metal ions and the reducing agent; and

incubating the enzyme with the metal ions in the presence of the oxidizing agent and the reducing agent.

85. (Previously Presented) The method of claim 69, wherein the step of depositing includes depositing the elemental metal within about 1 micron of the enzyme.

86. (Previously Presented) The method of claim 69, wherein the step of depositing includes depositing the elemental metal in the vicinity of the enzyme within a cell.

87. (Previously Presented) The method of claim 69, further comprising: localizing the enzyme in the area of a predetermined antigen.

88. (Previously Presented) The method of claim 87, wherein the predetermined antigen is a human cancer antigen.

89. (Previously Presented) The method of claim 87, wherein the predetermined antigen is a Her-2/neu protein.

90. (Previously Presented) The method of claim 69, further comprising: defining an antigen; and localizing the enzyme to the area of the defined antigen.

91. (Previously Presented) The method of claim 69, further comprising: localizing the enzyme in the area of a predetermined nucleic acid or nucleic acid probe.

92. (Previously Presented) The method of claim 91, wherein the predetermined nucleic acid is a Her-2/neu gene or a nucleic acid probe for a Her-2/neu gene.

93. (Previously Presented) The method of claim 69, further comprising: defining a nucleic acid probe; and binding the enzyme to the defined nucleic acid probe.

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94. (Previously Presented) The method of claim 69, further comprising: binding the enzyme to a member selected from antibody, antibody fragments, peptide, nucleic acids, nucleic acid probes, carbohydrates, drugs, steroids, products from plants, animals, humans and bacteria, and synthetic molecules, where each member has an affinity for binding to particular targets.
95. (Previously Presented) The method of claim 69, further comprising: binding an antibody to a predetermined antigen in a tissue section; and binding the enzyme to the antibody.
96. (Previously Presented) The method of claim 95, wherein the binding of the antibody to the enzyme is through biotin-avidin interaction.
97. (Previously Presented) The method of claim 96, wherein the antibody is a biotinylated monoclonal antibody; and the enzyme is conjugated with streptavidin.
98. (Previously Presented) The method of claim 95, wherein the tissue section is embedded in a solid support.
99. (Previously Presented) The method of claim 98, wherein the solid support is paraffin.
100. (Previously Presented) The method of claim 69, further comprising: binding a nucleic acid probe to a predetermined gene in the cells of a tissue section; and binding the enzyme to the nucleic acid probe.
101. (Previously Presented) The method of claim 100, wherein the binding of the nucleic acid probe to the enzyme is through biotin-avidin interaction.
102. (Previously Presented) The method of claim 101, wherein the nucleic acid probe is labeled with a fluorescent moiety; the enzyme is conjugated to streptavidin; and the binding of enzyme to the nucleic acid probe is through a biotinylated antibody against the fluorescent moiety.
103. (Previously Presented) The method of claim 69, wherein the metal ions are silver ion; and the method further comprises pretreating the enzyme with gold ions prior to the step of incubating.

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104. (Previously Presented) The method of claim 103, wherein the step of pretreating includes washing away residual gold ions prior to the step of incubating.

105. (Previously Presented) The method of claim 69, wherein the enzyme is horseradish peroxidase; the metal ions are silver ion; the oxidizing agent is hydrogen peroxide; and the reducing agent is hydroquinone.

106. (Previously Presented) The method of claim 69, wherein the step of incubating includes incubating the enzyme with the metal ions in the presence of the oxidizing agent and the reducing agent in a controlled pH buffer solution.

107. (Previously Presented) The method of claim 106, wherein the controlled pH buffer solution is a citrate buffer at about pH 3.8.

108. (Previously Presented) The method of claim 69, further comprising: stopping the deposition of the elemental metal to the vicinity of the enzyme after a certain period of time.

109. (Previously Presented) The method of claim 108, wherein the step of stopping includes washing away residual metal ions from the enzyme.

110. (Previously Presented) The method of claim 69, further comprising: detecting the elemental metal deposited in the vicinity of the enzyme.

111. (Previously Presented) The method of claim 69, further comprising: detecting the elemental metal deposited in the vicinity of the enzyme by automatallography.

112. (New) A kit for depositing elemental metal in the vicinity of an enzyme, comprising: metal ions selected from the group consisting of silver, gold, iron, mercury, nickel, copper, platinum, palladium, cobalt, iridium ions and a mixture thereof; an oxidizing agent; a reducing agent; an enzyme; and instructions for how to perform the method of claim 69.

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113. (New) The kit of claim 112, further comprising: a binding moiety that binds to a target molecule.

114. (New) The kit of claim 113, wherein the binding moiety is selected from the group consisting of antibody, antibody fragments, peptide, nucleic acids, nucleic acid probes, carbohydrates, drugs, steroids, products from plants, animals, humans and bacteria, and synthetic molecules, where each member has an affinity for binding to the target molecule.

115. (New) The kit of claim 114, wherein the target molecule is a target gene or genome and the binding moiety is a nucleic acid probe that binds to the target gene or genome.

116. (New) The kit of claim 115, wherein the target gene is Her-2/neu gene.

117. (New) The kit of claim 115, wherein the nucleic acid probe is labeled with biotin.

118. (New) The kit of claim 114, wherein the target molecule is a target antigen and the binding moiety is a primary antibody predetermined to bind to the target antigen.

119. (New) The kit of claim 118, wherein the target antigen is selected from the group consisting of Her-2/neu protein, estrogen receptor, and progesterone receptor.

120. (New) The kit of claim 118, wherein the enzyme is conjugated to a secondary antibody that binds to the primary antibody.

121. (New) The kit of claim 112, wherein the enzyme is peroxidase; the metal ions are silver ion; the oxidizing agent is hydrogen peroxide; and the reducing agent is hydroquinone.

122. (New) The kit of claim 121, wherein the enzyme is horseradish peroxidase; and the metal ions are silver acetate.

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123. (New) A kit for detecting Her-2/neu gene or protein in a test sample, comprising:  
metal ions selected from the group consisting of silver, gold, iron, mercury, nickel, copper, platinum, palladium, cobalt, iridium ions and a mixture thereof; an oxidizing agent; a reducing agent; an enzyme; and a binding moiety that binds to Her-2/neu gene or protein in the test sample.
124. (New) The kit of claim 123, wherein the binding moiety is a nucleic acid probe predetermined to bind to Her-2/neu gene.
125. (New) The kit of claim 124, wherein the nucleic acid probe is labeled with biotin.
126. (New) The kit of claim 123, wherein the moiety is a primary antibody predetermined to bind to the Her-2/neu protein.
127. (New) The kit of claim 126, wherein the enzyme is conjugated to a secondary antibody that binds to the primary antibody.
128. (New) The kit of claim 123, wherein the enzyme is peroxidase; the metal ions are silver ion; the oxidizing agent is hydrogen peroxide; and the reducing agent is hydroquinone.
129. (New) A kit for depositing elemental metal in the vicinity of an enzyme bound to a target molecule in a test sample, comprising:  
a test sample that comprises a target molecule; and is mixed with i) metal ions selected from the group consisting of silver, gold, iron, mercury, nickel, copper, platinum, palladium, cobalt, iridium ions and a mixture thereof; ii) an oxidizing agent; iii) a reducing agent; and iv) an enzyme, wherein the enzyme binds to the target molecule and reduces the metal ions to elemental metal in the presence of the oxidizing agent and reducing agent.

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130. (New) The kit of claim 129, wherein the test sample further comprises a nucleic acid probe that binds to the target molecule.

131. (New) The kit of claim 130, wherein the target molecule is a target gene or genome; and the nucleic acid probe is a probe for the target gene or genome.

132. (New) The kit of claim 131, wherein the target gene is Her-2/neu gene.

133. (New) The kit of claim 132, wherein the nucleic acid probe is labeled with biotin.

134. (New) The kit of claim 129, wherein the enzyme binds to the target molecule via a primary antibody that binds to the target molecule.

135. (New) The kit of claim 134, wherein the enzyme is conjugated to a secondary antibody that binds to the primary antibody.

136. (New) The kit of claim 129, wherein the enzyme is peroxidase; the metal ions are silver ion; the oxidizing agent is hydrogen peroxide; and the reducing agent is hydroquinone.